I. PATENTABILITY ARGUMENTS

A. Claim Amendments Submitted Herewith Do Not Introduce Any New Matter.

Applicant amends claims 145, 78, 80, 82 and 84 to clarify that enzyme or fragment thereof has enzymatic activity when displayed at the surface of filamentous bacteriophage. This amendment does not introduce any new matter and is fully supported by the instant specification as filed.

B. The Rejection Under 35 U.S.C. § 102(b) Should Be Withdrawn.

The Examiner rejected claims 46, 48-65, 78-109 and 145 under 35 U.S.C. § 102(b) as allegedly being anticipated by EP 0436597 B1 (Ladner EP). The rejection should be withdrawn because as submitted below, Ladner EP fails to teach each and every element of the instant invention and also fails to enable the practice of the present invention and in fact demonstrably fails to enable the practice of its own alleged invention. As a preliminary matter, the Applicants wish to point out that contrary to the Examiner's assertion set out in section I.C. below the Ladner EP and LADNER US are very different disclosures.

In order to properly anticipate a claim a reference must, *inter alia*, enable one skilled in the art to practice the claimed invention. *Titanium Metals Corp. of America v. Banner*, 778 F.2d 775, 778, 227 U.S.P.Q. 773 (Fed.Cir., 1985). "A reference is not an anticipation which does not enable one skilled in the art to practice the claimed invention."

The issue of enablement of Ladner EP was addressed at the European Patent Office (EPO) Board of Appeal (the "Board") (the highest level of technical review at the European Office and not subject to further review) in its decision T0792/00 ("Reasons for Decision") which affirmed the completely non-enabling nature of the Ladner EP disclosure even with

respect to its narrowest claim (claim 29) let alone a claim covering the display of enzymes which have enzymatic activity as claimed in the present application. A copy of the decision is attached hereto for the Examiner's convenience. While Applicants appreciate that the EPO is not binding authority in the U.S., the decision and analysis set out in T0792/00 is informative as to why Ladner EP fails to enable a person of ordinary skill in the to practice the alleged invention as disclosed by Ladner and thus fails to enable the practice of the present invention.

As a matter of first principles, Applicants point out that Ladner EP contains no experimental data nor does it demonstrate any successful operation of the disclosed technology. Indeed, the disclosure itself repeatedly admits to the possibility and likelihood of failure (see, *e.g.,* Ladner EP at page 25, lines 7, 29 and 45; page 45, lines 10-18; and from page 49, lines 35 to line 52, which points to various sources of possible failure in attempts to practice their alleged invention. In addressing the issue of failure, the Reasons for Decision states at paragraph 24:

> **In other words, the patent in suit itself casts strong doubts on the possibility to perform the claimed object.** Furthermore, since every element of the solution proposed in the patent in suit (i.e., signal sequence, outer surface protein, genetic package) may be, according to the sentence of the patent in suit (page 52, lines 7 to 9), a potential source of failure, the patent in suit does not provide the skilled person with a real guidance to perform the claimed subject-matter but on the contrary, in the Board's view, offers nothing else to the skilled person than an outline of a search programme. An invention, however, is supposed to relate to a solution to a technical problem. First to perform a research programme that the patentee has outlined but not himself performed, and for which the prospects of success appear poor, is not a burden that can be put on a skilled person trying to reproduce an invention. (Emphasis added.)

With respect to the hypothetical example of Ladner EP, which is limited to a small non-enzymatic molecule bovine trypsin inhibitor (BPTI) considered to be the protein specifically

selected by Ladner EP because it had properties most likely to succeed, the Board wrote at paragraph 17 of the Reasons for Decision:

> Referring to the description, the skilled person will find a single example, Example I, using BPTI-derived binding protein to be displayed on an M13 phage. The example emphasizes throughout that it only gives a hypothetical example of a protocol. From reading the example alone the skilled reader cannot derive any certainty that the invention claimed in claim 29 can be got to work according to protocol.

At paragraph 25 of its Reasons for the Decision, the Board also stated that:

> In the hypothetical protocol the protein used is BPTI stated to be chosen because it is a small, very stable protein with a well known 3D structure (see page 127 of application as filed). If the skilled person would not have succeeded with this, where the chances of success seemed better than for anything else, the only likely conclusion he would draw is that the patent specification does not contain sufficient information to carry out the subject matter of claim 29, if this can be carried out at all.

Thus, the Applicants respectfully submit that disclosure of Ladner EP is wholly non-enabling of a display of any folded protein domain even as a small and stable as BPTI a 58 amino acid protein with no enzymatic activity.

Further, Ladner EP also fails to disclose or enable the presently claimed combination of the display of an **active** enzyme or fragment thereof on a filamentous phage. As discussed in more detail in Section C below, Sec. 2.3 on page 21 of Ladner EP states:

> If the chosen IPBD is an enzyme, it may be necessary to change one or more residues in the active site to inactive enzyme function. For example, if the IPBD were T4 lysozyme, and the GP were *E.coli,* cells or M13 we would inactivate the enzyme lest it lyse the cells.

It is important to note that M13 is a filamentous phage (which is experimentally exemplified in the present application) and thus the teaching of Ladner EP even if enabled does

not teach display of an active enzyme on the surface of a filamentous phage, but rather, an inactivated enzyme.

Because Ladner teaches inactivating an enzyme when displayed on a filamentous phage and because the present claims recite the display of an active enzyme or fragment on filamentous phage, Ladner EP cannot properly anticipate the present invention.

In summary, because Ladner EP is not enabling for display of anything and because it fails to disclose all of the elements of the present claims, that is, the display of an active enzyme on a filamentous phage, it cannot properly anticipate the instant invention. Therefore, the rejection of claims 46, 48-65, 78-109 and 145 under 35 U.S.C. § 102 (b) should be withdrawn.

C. The Rejections Under 35 U.S.C. § 102 (e) Should Be Withdrawn.

The Examiner has rejected claims 46, 48-65, 78-109 and 145 under 35 U.S.C. § 102(e) as allegedly being anticipated by US Patent 5,223,409 (LADNER US).

The claims of the LADNER US patent are no bar to patentability of the presently claimed invention because *inter alia* the patent fails to teach or claim the specific combination of features presently claimed and therefore the rejections should be withdrawn.

In particular, there is no specific disclosure of the combination of display of an enzyme or fragment thereof with enzymatic activity on the surface of filamentous bacteriophage. As discussed further below, LADNER US contains no experimental exemplification of display of any enzyme and teaches a potpourri of elements to choose from such as various different kinds of genetic packages for display (cells, spores and different kinds of viruses) teaches inactivation of enzyme activity if filamentous bacteriophage are used, and does not teach the specific combination of features claimed in the present application.

The Applicants submit the present invention as claimed prior to the present amendment is patentable over LADNER US. However, to expedite prosecution a clarifying amendment is made herein, reciting that the enzyme or fragment thereof has enzymatic activity when displayed on the surface of filamentous bacteriophage particles, an element not taught by LADNER US. Support for this amendment is found throughout the specification, especially as provided by the experimental examples.

LADNER US is concerned with methods that involve use of an "IPBD" – "Initial Potential Binding Domain" – to search for binding proteins with binding properties relative to the target that are superior to the initial binding properties. See, *e.g.,* LADNER US, column 11, and throughout the specification.

Column 16 of LADNER US defines "binding protein" (BP) and "initial potential binding domain" (IPBD), which is what is to be displayed and subject to variegation:

> When a domain of a protein is primarily responsible for the protein's ability to specifically bind a chosen target, it is referred to herein as a "binding domain" (BD). A preliminary operation is to engineer the appearance of a stable protein domain, denoted as an "initial potential domain" (IPBD), on the surface of a genetic package.

See also, column 18

IPBD	Initial Potential Binding Domain, *e.g.,* BPTI
PBD	Potential Binding Domain, *e.g.,* a derivative of BPTI
SBD	Successful Binding Domain, *e.g.*, a derivative of BPTI Selected for binding to a target
PPBD	Parental Potential Binding Domain, *i.e.,* an IPBD or an SBD from a previous selection.

Column 20 of LADNER US discusses the selection of IPBD:

> Preferably, the IPBD is no larger than necessary because small SBDs (for example, less than 30 amino acids) can be chemically synthesized and because it is easier to arrange restriction sites in smaller

> amino-acid sequences. For PBDs smaller than about 40 residues, an added advantage is that the entire variegated pbd gene can be synthesized in one piece. In that case, we need arrange only suitable restriction sites in the osp gene. A smaller protein minimizes the metabolic strain on the GP or the host of the GP. The IPBD is preferably smaller than about 200 residues. The IPBD must also be large enough to have acceptable binding affinity and specificity. For an IPBD lacking covalent crosslinks, such disulfide bonds, the IPBD is preferably at least 40 residues; it may be as small as six residues if it contains a crosslink. These small, crosslinked IPBDs, known as "mini-proteins", are discussed in more detail later in this section.

Here there is emphasis on the IPBD being " no larger than necessary" with small domains being preferred. There is an explicit statement "The IPBD is preferably smaller than about 200 residues." This paragraph is further qualified by the following paragraph that describes various potential properties to be taken into account:

> Some candidate IPBDs, which meet the conditions set forth above, will be more suitable than others. Information about candidate IPBDs that will be used to judge the suitability of the IPBD includes: 1) a 3D structure (knowledge strongly preferred), 2) one or more sequences homologous to the IPBD (the more homologous sequences known, the better), 3) the pI of the IPBD (knowledge desirable when target is highly charged), 4) the stability and solubility as a function of temperature, pH and ionic strength (preferably known to be stable over a wide range and soluble in conditions of intended use), 5) ability to bind metal ions such as CA^{++} or Mg^{++} (knowledge preferred; binding *per se*, no preference), 6) enzymatic activities, if any (knowledge preferred, activity per se has uses but may cause problems), 7) binding properties, if any (knowledge preferred, specific binding also preferred), 8) availability of a molecule having specific and strong affinity ($K_d<10^{-11}M$) for the IPBD (preferred), 9) availability of a molecule having specific and medium affinity ($10^{-8}M<K_d<10^{-6}M$) for the IPBD (preferred), 10) the sequence of a mutant of IPBD that does not bind to the affinity molecule(s) (preferred), and 11) absorption spectrum in visible, UV, NMR, etc., (characteristic absorption preferred).

Thus, while the generic disclosure within LADNER US teaches a preference for small, stable protein domains (but does entertain the possibility of larger ones), there is no explicit disclosure

of display of large (*e.g.*, greater than 100 amino acids or greater than 200 amino acid) protein domains on the surface of filamentous bacteriophage (as a GP), and no explicit disclosure of enzymes or fragments thereof on the surface of filamentous bacteriophage where the enzymes are enzymatically active.

Column 21 of LADNER US mentions candidate IPBDs, including the enzyme lysozyme:

> There are many candidate IPBDs for which all of the above information is available or is reasonably practical to obtain, for example, bovine pancreatic tyrpsin inhibitor (BPTI, 58 residues), CMTI-III (29 residues), crambin (46 residues), third domain of ovomucoid (56 residues), heat-stable enterotoxin (ST-Ia of *E.coli*) (18 residues), α-Conotoxin GI (13 residues), μ-Conotoxin GIII (22 residues), Conus King Kong mini-protein (27 residues), T4 lysozme (1654 residues), and azurin (128 residues).

However, in column 23, LADNER US specifically discusses a problem with enzymes and in particular with an enzyme displayed on the filamentous phage M13:

> II.D. Other consideration in the choice of IPBD:
>
> If the chosen IPBD is an enzyme, it may be necessary to change one or more residues in the active site to inactive enzyme function. For example, if the IPBD were T4 lysozyme and the GP were *E.coli* cells or M13, we would to inactivate the lysozyme because otherwise it would lyse the cells. If, on the other hand, the GP were ΦX174, then inactivation of lysozyme may not be needed because T4 lysozyme can be overproduced inside *E.coli* cells without detrimental effects and ΦX174 forms intracellularly. It is preferred to inactivate enzyme IPBDs that might be harmful to the GP or its host by substituting mutant amino acids at one or more residues of the active site. It is permitted to vary one or more of the residues that were changed to abolish the original enzymatic activity of the IPBD. Those GPs that receive osp-pbd genes encoding an active enzyme may die, but the majority of sequences will not be deleterious.

Here, the LADNER US talks about inactivating lysozyme if using M13, a filamentous phage for display. The present claims are limited to the use of filamentous phage and to

the display of an active enzyme or fragment thereof. Experimental examples in the present application actually demonstrate display of active enzymes on the surface of filamentous bacteriophage particles. Ladner does discuss the possibility that an enzyme need not be inactivated if using ΦX174, which is not a filamentous phage as is presently claimed.

Because, *inter alia*, Ladner suggests the display of *inactive* enzymes on the surface of filamentous phage, while the present claim recites the display of enzymes or fragments thereof which have enzymatic activity on the filamentous phage and because it fails to teach the specific combination of elements presently claimed but rather discusses a menu of options, the Applicants submit that LADNER US cannot properly anticipate the present claims and therefore that the rejections under 35 U.S.C. § 102(e) should be withdrawn.

D. The Rejections Under 35 U.S.C. § 103(a) Should Be Withdrawn.

The Examiner rejected claims 46, 48-65, 78-109 and 145 under 35 U.S.C. § 103(a) as allegedly being obvious over Ladner EP or LADNER US. The Examiner also alleges that the disclosure of LADNER EP is the same as the one of LADNER US. The Applicants respectfully submit that the rejection should be withdrawn because *inter alia* Ladner EP is non-enabling (and may not properly serve as a reference against the present application) and because LADNER US actually teaches away from the present invention and fails to provide the requisite expectation of success necessary to find the present invention obvious.

Applicants reiterate that Ladner EP and LADNER US, contrary to the Examiner's assertion, are two different disclosures because Ladner EP claims priority to US 07/240,160, and LADNER US has a different relationship with that application:

The front page of the LADNER US states:

> Continuation-in-part of Ser. No. 487,063, Mar. 2, 1990, abandoned, and a continuation-in-part of Ser. No. 240,160, Sep. 2, 1988, abandoned.

The first paragraph of the description states:

> This application is a continuation-in-part of Ladner, Guterman, Roberts, and Markland, Ser. No. 07/487,063, filed Mar. 2, 1990, now abandoned, which is a continuation-in-part of Ladner and Guterman, Ser. No. 07/240,160, filed Sep. 2, 1988, now abandoned. Ser. No. 07/487,063 claimed priority under 35 U.S.C. 119 from PCT Application No. PCT/US89/03731, filed Sep. 1, 1989. All of the foregoing applications are hereby incorporated by reference.

The CIP (of a CIP) status of the LADNER US is significant: two times subject-matter was added to the LADNER US disclosure which does not appear in the LADNER EP (PCT). The added subject-matter has effective dates of 2 March 1990 and 1 March 1991. Thus, LADNER US and Ladner EP are two different disclosures.

As discussed in section I.C. above, the Ladner EP disclosure is totally hypothetical devoid of experimental results non-enabling and therefore, cannot properly serve as a proper obviousness reference against the present application because references not available for purposes of 35 USC § 102 are also not available under 35 USC § 103. Further, the Applicants respectfully submit that LADNER US (which contains experimental results limited to small non-enzyme molecules) actually teaches away from the present invention in numerous parts of the specification and therefore cannot render the present invention obvious. Further, LADNER US does nothing to overcome the prejudice in the art against expressing functional polypeptides over 100 amino acids on the surface of phage.

In teaching away from the present invention, LADNER US discusses the display of enzymes, in column 23, and specifically identifies a problem with the display of enzymes:

The reference states at Column 23:

> II.D. Other consideration in the choice of IPBD:
>
> > If the chosen IPBD is an enzyme, it may be necessary to change one or more residues in the active site to inactive enzyme function. For example, if the IPBD were T4 lysozyme and the GP were *E.coli* cells or M13, we would need lyse the cells. If, on the other hand, the GP were ΦX174, then inactivation of lysozyme may not be needed because T4 lysozyme can be overproduced inside *E.coli* cells without detrimental effects and ΦX174 forms intracellularly. It is preferred to inactivate enzyme IPBDs that might be harmful to the GP or its host by substituting mutant amino acids at one or more residues of the active site. It is permitted to vary one or more of the residues that were changed to abolish the original enzymatic activity of the IPBD. Those GPs that receive osp-pbd genes encoding an active enzyme may die, but the majority of sequences will not be deleterious.

As discussed above and reiterated here for the Examiner's convenience, LADNER US provides specific teaching in relation to the different combination of enzyme and genetic package (GP). Specifically, where the GP is to be *E.coli* cells or M13 (a filamentous phage) "we would need to inactivate the lysozyme", whereas a different GP ΦX174 (not a filamentous phage) "inactivation may not be needed".

> Preferred GPs are vegetative bacterial cells, bacterial spores and bacterial DNA viruses. Eurkaryotic cells could be used as genetic packages but have longer dividing time and more stringent nutritional requirements than do bacteria and it is much more difficult to produce a large number of independent transformants. They are also more fragile than bacterial cells and therefore more difficult to chromatograph without damage. Eurkaryotic viruses could be used instead of bacteriophage but must be propagated in eukaryotic cells and therefore suffer from some of the amplification problems mentioned above.

As noted above, the experiments first described in LADNER US employ BPTI, a small very stable non-enzymatic protein chosen precisely because it is small and very stable. Columns 23 and 24 of LADNER US state:

> BPTI is an especially preferred IPBD because it meets or exceeds all the criteria: it is a small, very stable protein with a well known 3D structure. Marks *et al.* (MARKS86) have shown that a fusion of the phoA signal peptide gene fragment and DNA coding for the mature form of BPTI caused native BPTI to appear in the periplasm of *E.coli* demonstrating that there is nothing in the structure of BPTI to prevent its being secreted.
>
> The structure of BPTI is maintained even when one or another of the disulfides is removed, either by chemical blocking or by genetic alteration of the amino-acid sequence. The stabilizing influence of the disulfides in ...
>
> ...crystal lattices. This indicates that BPTI is redundantly stable and is so likely to fold into approximately the same structure despite numerous surface mutations.
>
> BPTI has been called "the hydrogen atom of protein folding" and has been the subject of numerous experimental and theoretical studies (STAT87, SCHW87, GOLD83, CHAZ83, CRE174, CRE177a, CRE177b, CRE180, SIEK87, SINH90, RUEH73, HUBE74, HUBE75, HUBE77 and others).
>
> BPTI is freely soluble and is not known to bind metal ions. BPTI has no known enzymatic activity. BPTI is not toxic.

The fact that "BPTI has no known enzymatic activity" is a characteristic taken into account in its selection as a candidate for display. The fair inference from this is that less stable proteins such as enzymes are not likely to be successfully displayed on the surface of filamentous phage.

Columns 25-26 of LADNER US again discuss the advantages of using small proteins, noting some disadvantages that are then said to be overcome using the disclosed mini-proteins,

the desire being to capitalize on the desirable advantages of small proteins while eliminating the undesirable disadvantages.

Additional problems are identified in LADNER US where in column 54 it discusses problems with viability and states:

> Bacteriophages are excellent candidates for GPs because there is little or no enzymatic activity associated with intact mature phage, and because the genes are inactive outside a bacterial host, rendering the mature phage particles metabolically inert.
>
> The filamentous phages (*e.g.*, M13) are of particular interest.
>
> For a given bacteriophage, the preferred OSP is usually one that is present on the phage surface in the largest number of copies, as this allows the greatest flexibility in varying the ratio of OSP-IPBD to wild type OSP and also gives the highest likelihood of obtaining satisfactory affinity separation. Moreover, a protein present in only one or a few copies usually performs an essential function in morphogenesis or infection; mutating such a protein by addition or insertion is likely to result in reduction in viability of the GP. Nevertheless, an OSP such as M13 gIII protein may be an excellent choice as OSP to cause display of the PBD.

Thus, aside from the question of whether it would be possible to display an IPBD in a functional, binding form, there were likely to be problems with reduction in viability of the GP, again mitigating against the likelihood of success required to render an invention obvious.

Another factor mitigating against the necessary expectation of success required to find an invention obvious, is the prejudice in the art existing at the time the present invention was made against the display of functional polypeptides over 100 amino acids on the surface of the filamentous phage a prejudice that Ladner EP and LADNER US fails to overcome in that the references never demonstrated that it was in fact possible to display functional polypeptides of that size. Specifically, prior to the present invention, there was a prejudice in the art that it was

not possible to display, at the surface of a phage particle, a polypeptide that is larger than about 100 amino acids and which was functional. For example, those of skill in the art recognized that the largest size of fragment for display on a filamentous phage should be 100-300 bp (corresponding to 33-100 amino acids). (See Parmley and Smith, *Gene* 73, 305-318 (1988) at p. 315 of record and discussed throughout early prosecution of this application). Others stated that "...[I]t has been shown that fusion phage are useful only for displaying proteins of less than 100 and preferably less than 50 residues, because large inserts presumably disrupt the function of gene III, and therefore phage assembly and infectivity..." Bass *et al., Protein: Structure, Function & Genetics, 8*, 309-314 (1990) (citing Parmley and Smith, *Gene*, 73, 305-318 (1988) also disclosed earlier in the prosecution of this application). See also Smith Grant (*Smith Grant Application, entitled: "Filamentous Phage Physiology", dated November 1, 1988* and *Abstract of George P. Smith, Grant No. 1R01GM41478-01A1, Project Start Date: July 1, 1989)*, also of record, which contains no experimental demonstration of display of large inserts on the surface of phage, and, while suggesting an attempt to be made at displaying single-chain antibody molecules (not non-immunoglobulin active enzymes as in the present invention), highlight a number of expected difficulties and problems, consistent with the Parmley and Smith and Bass papers already mentioned. Furthermore, there was no expectation of being able to display a functional, folded protein domain, especially such a domain of larger than 100 amino acids. The present invention overcame the prejudice in the art by providing, for the first time, an enabling disclosure and specific working examples of the bacteriophage display of functional protein domains having at least 100 amino acids including, for the first time, the display of active enzymes.

In view of the non-enabling nature of Ladner EP, the teaching against the display of enzymes having enzymatic activity on the surface of filamentous phage as discussed in Ladner EP and LADNER US further in view of the failure of either reference to overcome the prejudice in the art against display of functional polypeptides of over 100 amino acids on phage and in view of the references failure to provide the requisite expectation of success, the Applicants respectfully submit that neither Ladner EP nor LADNER US can properly render the present invention obvious and therefore the rejections should be withdrawn.

Conclusion.

In view of the foregoing amendments and remarks, the applicants respectfully submit that the claims are now in condition for allowance and early notification thereof is earnestly solicited.

Respectfully submitted,

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